CLAIMS

WHAT IS CLAIMED IS:

1. A method for amplifying a target nucleotide sequence or reverse complement thereof, the method comprising:

providing a template nucleic acid comprising a first strand, the first strand comprising a target region that comprises the target nucleotide sequence or its reverse complement;

providing a first linear primer comprising a region of identity to a 5' subregion of the target region;

providing a second linear primer comprising a region of complementarity to a 3' subregion of the target region;

providing a hairpin primer comprising a region of complementarity to a first subregion of the target region, the first subregion being 5' of or at least partially overlapping the 3' subregion;

contacting the template nucleic acid, the first linear primer, the second linear primer and the hairpin primer; and,

extending at least the hairpin primer, thereby amplifying at least a portion of the target nucleotide sequence or its reverse complement.

2. The method of claim **1**, wherein the hairpin primer comprises a 5' arm, a loop and a 3' arm, the 5' arm and the 3' arm being complementary to each other and able to form a double-stranded duplex, and at least a portion of the loop and the 3' arm being complementary to the first subregion of the target region.

3. The method of claim **2**, wherein the hairpin primer consists of a 5' arm, a loop and a 3' arm, the 5' arm and the 3' arm being complementary to each other and able to form a double-stranded duplex, and at least a portion of the loop and the 3' arm being complementary to the first subregion of the target region.

4. The method of claim **2**, wherein the entire loop and 3' arm are complementary to the first subregion.

5. The method of claim **2**, wherein the hairpin primer comprises a fluorescent label that emits a fluorescent signal and a quencher, the label and the quencher being located within the hairpin primer such that the label emits a maximal fluorescent signal only when the 5' and 3' arms are not forming the double-stranded duplex.

6. The method of claim **5**, wherein incorporation of the hairpin primer into a double-stranded product of the extending step prevents formation of the double-stranded duplex and thus permits emission of the maximal fluorescent signal.

7. The method of claim **5**, comprising detecting the fluorescent signal emitted by the label.

8. The method of claim **7**, wherein detecting the fluorescent signal comprises measuring the intensity of the fluorescent signal.

9. The method of claim **5**, wherein:

providing a first linear primer comprises providing two or more first linear primers, each of which comprises a region of identity to a 5' subregion of a different target region;

providing a second linear primer comprises providing two or more second linear primers, each of which comprises a region of complementarity to a 3' subregion of a different target region;

providing a hairpin primer comprises providing two or more hairpin primers, each of which comprises a region of complementarity to a first subregion of a different target region, the first subregion being 5' of or at least partially overlapping the 3' subregion of that target region;

providing a template nucleic acid comprises providing one or more template nucleic acids collectively comprising the different target regions; and,

extending at least the hairpin primer comprises extending at least each of the hairpin primers.

10. The method of claim **9**, wherein each hairpin primer comprises a different fluorescent label that emits a fluorescent signal distinguishable from that of each of the other fluorescent labels, the method comprising detecting the fluorescent signal from the label on each of the two or more hairpin primers.

11. The method of claim **1**, comprising extending the first and second linear primers.

12. The method of claim **1**, wherein the first linear primer is provided a concentration that is at least about 1.3 times that of the second linear primer.

13. The method of claim **12**, wherein the first linear primer is provided a concentration that is at least about two times that of the second linear primer.

14. The method of claim **12**, wherein the first linear primer is provided a concentration that is at least about three times that of the second linear primer.

15. The method of claim **1**, wherein the first subregion at least partially overlaps the 3' subregion.

16. The method of claim **1**, wherein the first subregion does not overlap the 3' subregion.

17. The method of claim **1**, wherein extending at least the hairpin primer comprises using a polymerase substantially lacking 5' to 3' nuclease activity to extend at least the hairpin primer.

18. The method of claim **1**, wherein the hairpin primer is resistant to 5' to 3' nuclease activity.

19. The method of claim **1**, wherein the template nucleic acid is a single-stranded DNA product of a reverse transcription reaction.

20. The method of claim **1**, wherein the template nucleic acid is a double-stranded cDNA, a single-stranded PCR product, or a double-stranded PCR product.

21. The method of claim **1**, wherein the template nucleic acid comprises genomic DNA.

22. A composition comprising:

a template nucleic acid comprising a first strand, the first strand comprising a target region that comprises a target nucleotide sequence or its reverse complement;

a first linear primer comprising a region of identity to a 5' subregion of the target region;

a second linear primer comprising a region of complementarity to a 3' subregion of the target region; and,

a hairpin primer comprising a region of complementarity to a first subregion of the target region, the first subregion being 5' of or at least partially overlapping the 3' subregion.

23. The composition of claim **22**, wherein the hairpin primer comprises a 5' arm, a loop and a 3' arm, the 5' arm and the 3' arm being complementary to each other and able to form a double-stranded duplex, and at least a portion of the loop and the 3' arm being complementary to the first subregion of the target region.

24. The composition of claim **23**, wherein the hairpin primer consists of a 5' arm, a loop and a 3' arm, the 5' arm and the 3' arm being complementary to each other and able to form a double-stranded duplex, and at least a portion of the loop and the 3' arm being complementary to the first subregion of the target region.

25. The composition of claim **23**, wherein the entire loop and 3' arm are complementary to the first subregion.

26. The composition of claim **23**, wherein the hairpin primer comprises a fluorescent label that emits a fluorescent signal and a quencher, the label and the quencher being located within the hairpin primer such that the label emits a maximal fluorescent signal only when the 5' and 3' arms are not forming the double-stranded duplex.

27. The composition of claim **26**, wherein the hairpin primer is extended to form a double-stranded product, such incorporation of the hairpin primer into the product preventing formation of the double-stranded duplex and thus permitting emission of the maximal fluorescent signal.

28. The composition of claim **22**, wherein the first linear primer is provided a concentration that is at least about 1.3 times that of the second linear primer.

29. The composition of claim **28**, wherein the first linear primer is provided a concentration that is at least about two times that of the second linear primer.

30. The composition of claim **28**, wherein the first linear primer is provided a concentration that is at least about three times that of the second linear primer.

31. The composition of claim **22**, wherein the first subregion at least partially overlaps the 3' subregion.

32. The composition of claim **22**, wherein the first subregion does not overlap the 3' subregion.

33. The composition of claim **22**, comprising a polymerase.

34. The composition of claim **33**, wherein the polymerase substantially lacks 5' to 3' nuclease activity.

35. The composition of claim **22**, wherein the hairpin primer is resistant to 5' to 3' nuclease activity.

36. The composition of claim **22**, wherein the template nucleic acid is a single-stranded DNA product of a reverse transcription reaction.

37. The composition of claim **22**, wherein the template nucleic acid is a double-stranded cDNA, a single-stranded PCR product, or a double-stranded PCR product.

38. The composition of claim **22**, wherein the template nucleic acid comprises genomic DNA.

39. The composition of claim **22**, wherein the composition is contained in a thermal cycler.

40. A kit for use in amplifying a target nucleotide sequence or reverse complement thereof from a template nucleic acid strand comprising a target region that comprises the target nucleotide sequence or its reverse complement, the kit comprising:

a first linear primer comprising a region of identity to a 5' subregion of the target region;

a second linear primer comprising a region of complementarity to a 3' subregion of the target region; and,

a hairpin primer comprising a region of complementarity to a first subregion of the target region, the first subregion being 5' of or at least partially overlapping the 3' subregion,

packaged in one or more containers.

41. The kit of claim **40**, wherein the hairpin primer comprises a 5' arm, a loop and a 3' arm, the 5' arm and the 3' arm being complementary to each other and able to form a double-stranded duplex, and at least a portion of the loop and the 3' arm being complementary to the first subregion of the target region.

42. The kit of claim **41**, wherein the hairpin primer consists of a 5' arm, a loop and a 3' arm, the 5' arm and the 3' arm being complementary to each other and able to form a double-stranded duplex, and at least a portion of the loop and the 3' arm being complementary to the first subregion of the target region.

43. The kit of claim **41**, wherein the entire loop and 3' arm are complementary to the first subregion.

44. The kit of claim **41**, wherein the hairpin primer comprises a fluorescent label that emits a fluorescent signal and a quencher, the label and the quencher being located within the hairpin primer such that the label emits a maximal fluorescent signal only when the 5' and 3' arms are not forming the double-stranded duplex.

45. The kit of claim **40**, wherein the first subregion at least partially overlaps the 3' subregion.

46. The kit of claim **40**, wherein the first subregion does not overlap the 3' subregion.

47. The kit of claim **40**, wherein the hairpin primer is resistant to 5' to 3' nuclease activity.

48. The kit of claim **40**, comprising one or more of: a polymerase, a buffer, a standard template for calibrating a detection reaction, instructions for extending the hairpin primer to amplify at least a portion of the target nucleotide sequence or reverse complement thereof, instructions for using the components to amplify, detect and/or quantitate the target nucleotide sequence or reverse complement thereof, or packaging materials.